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13. ABSTRACT (Maximum 200 words)

During the past year Dr. Alan I. Faden was named Institute Director and aggressive recruitment of faculty was initiated. To date, nine additional new faculty appointments have been made. Fifteen postdoctoral fellows and four technicians have also been recruited, and an administrative support staff is in place that includes an administrator and medical secretary. Laboratory renovations have been performed at Georgetown's expense ($692,184) to accommodate the new faculty in an optimal research environment; these include a new research suite in the basement of the New Research Building to house the 7.0 Tesla nuclear magnetic resonance machine, and associated surgical and physiological monitoring space. Equipment has been ordered for the new faculty, who have continued productive research during the transition period by collaborating with their prior laboratories. The cognitive and brain imaging components of the program are now well-established. Recruitment is continuing for the computational component of the Institute, and an active recruitment schedule will continue until the Institute is fully staffed (approximately 15 faculty) by mid-1996.

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for Dr. Robert S. Ledley

11/9/95

PI - Signature Date

(For Georgetown Univ.)

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INTRODUCTION

The ability of existing computer systems to master the discrimination of patterns has been limited despite rapid increases in computational power. In contrast to learning abilities of the mammalian brain, computers are only able to acquire patterns that have first been learned and stored; following programming to accomplish this task, computers can collect and perform the necessary complex operations on generally narrowly specified types of patterns. Recent insights into the molecular and synaptic structure of brain networks have begun to be incorporated into the development of artificial computer-based networks. Such biologically-based pattern recognition principles permit the development of a new generation of computers than can, to a certain extent, be self-programming. Moreover, the exploration of biological networks, including parallel basic and applied research studies, will help to expand our knowledge of human brain functions and behavior.

DEVELOPMENT OF AN INSTITUTE FOR COGNITIVE AND COMPUTATIONAL SCIENCES

The Georgetown Institute for Cognitive and Computational Sciences (GICCS) has been established to study processes of pattern recognition and storage — including sensation, perception, learning and memory — from the molecular level to the operation of the complex brain networks. The Institute includes three research divisions. The Division of Cognitive Neuroscience focuses on the cellular and molecular mechanisms of perception, learning and memory, with particular reference to understanding the nature of biological networks, and ultimately, systems. The Division of Computational Neuroscience will emphasize development of artificial neural networks based on established biological principles. The Brain Imaging Division utilizes advanced and novel brain imaging techniques that relate both to the biologic and artificial

neural network components. The Institute also interfaces more broadly to other relevant neuroscience areas including electrophysiology (particularly relating to ion channels and receptors); molecular neurobiology (including signal transduction mechanisms); neuropharmacology (with particular reference to ligand receptor interactions and cognitive function); and, to computational physics and computer sciences.

Although the primary mission of the Institute is theoretical and experimental research, there is also an explicit commitment to develop relevant clinical and commercial applications that derive from these research efforts. Through this commitment, the Institute will serve as a catalyst for development and/or expansion of complementary research programs at the Medical Center and the Main Campus in such areas as neuroscience, developmental biology, computational physics, applied mathematics, and computer sciences. The Institute will also provide opportunities for graduate and undergraduate students who wish to pursue research in these areas.

An Internal Advisory Board has been established to contribute to the development of the Georgetown Institute for Cognitive and Computational Sciences and to serve as a Search Committee for new faculty. The Board has held three meetings thus far and will continue to meet on an ongoing basis to make recommendations concerning the goals and objectives related to the recruitment and scientific focus of the Institute. Its members include:

Stanley Cohan, M.D., Professor and Chair of the Department of Neurology;
Carol Colton, Ph.D., Associate Professor of Physiology and Biophysics;
Richard Goldberg, M.D., Professor and Chair of the Department of Psychiatry;
Darlene Howard, Ph.D., Professor of the Department of Psychology;
Robert Ledley, D.D.S., President and Director of the National Biomedical Research Foundation;
Michael Lumpkin, Ph.D., Professor and Acting Chair of the Department of Physiology and Biophysics;
Robert L. Martuza, M.D., Professor and Chair of the Department of Neurosurgery;
Joseph Neale, Ph.D., Professor and Chair of the Department of Biology;
Vassilios Papadopoulos, Ph.D., Assistant Professor of Cell Biology;
John Richert, M.D., Professor of the Department of Neurology;
Joseph Serene, Ph.D., Professor and Chair of the Department of Physics;
Sarah Spiegel, Ph.D., Associate Professor of the Department of Biochemistry and Molecular Biology; and
Robert Zeman, M.D., Professor and Clinical Director of the Division of Diagnostic Radiology.

Although Georgetown's original intent was to establish a Department of Neuroscience with the Chair also serving as Institute Director, the national search for an appropriate candidate proved overly lengthy and Georgetown University therefore appointed the Scientific Principal Investigator, Alan Faden, M.D. to serve as Director. The academic home for Institute faculty is a newly established Basic Neuroscience Division within the Department of Neurology; Dr. Faden, a Professor in the Department of Neurology, heads this division. Since his appointment, Dr. Faden has moved aggressively to recruit new faculty to form the core group for this new Institute. An extensive advertising program was conducted, with advertisements placed in *Science*, *Nature*, *Academic Medicine, Chemical and Engineering News*, and on the INTERNET to target appropriate scientists. A mass mailing was generated to solicit recommendation from Chairs of Departments of Neurology and Neuroscience nationwide. Recommendations were also solicited from scientific leaders in cognitive neuroscience, computational neuroscience, and brain imaging. A total of 144 applications were screened — 28 candidates were invited to make formal recruitment visits.

To date, nine faculty have been appointed, in addition to the Director. These include:

Josef Rauschecker, Ph.D. (Professor), Associate Director for GICCS;

Alan Kozikowski, Ph.D. (Professor);

James Pekar, Ph.D. (Assistant Professor), Director of the NMR Laboratory;

Rhonda Friedman, Ph.D. (Research Associate Professor);

Jian-Young Wu, Ph.D. (Assistant Professor);

Sheridan Swope, Ph.D. (Assistant Professor);

Rene Etcheberrigaray, Ph.D. (Research Assistant Professor);

Alexander G. Yakovlev, Ph.D, (Research Assistant Professor);

Biao Tian, Ph.D. (Research Associate)

Curricula vitae for these individuals are appended. Remaining recruitments will focus in the areas of auditory processing/pattern recognition; computational neuroscience (with some emphasis on the

auditory system); brain imaging, including human functional brain imaging related to learning and pattern recognition; and Alzheimer's Disease. The Institute has a highly competitive research faculty and recent recruitment visits have included individuals considered among the best in the country in their respective areas.

A permanent administrative staff has been appointed: Barbara Cohen-King, the Institute Administrator, has considerable prior experience in clinical/research neuroscience at the University of Rochester; Jennifer Cronan, previously employed at Integro Services-Sandoz Pharmaceuticals, has excellent secretarial experience, including manuscript preparation. To date, 15 postdoctoral fellows and four technicians have been hired to provide researchers with all appropriate support, with active recruitment continuing.

Laboratory space for new faculty is assigned within the New Research Building (NRB), (18,398 net square feet), the premium laboratory space at the Medical Center. Renovations have been made in the laboratory spaces at Georgetown's expense to accommodate the research program needs of the new faculty. This includes major construction of a new experimental NMR facility in the sub-basement of the NRB to house a 7.0 Tesla magnet, and associated surgical and monitoring space. This is expected on-line by early January 1996. Equipment has been ordered for the faculty now recruited, and experiments have begun in several laboratories. All faculty now on board should have fully operational laboratories within several months.

In order to enhance research productivity during this transitional period, all new faculty continue to collaborate with their previous laboratories while their new laboratories are being renovated and equipped. A program has also been initiated to support collaborative, joint research between existing Georgetown faculty and GICCS faculty. A total of 14 applications for this intramural program have been received, and a Peer Review Committee, chaired by Dr. Faden, will select appropriate projects.

In addition to the programs detailed above, Dr. Faden is establishing a "Center Without Walls," in which collaborating investigators from other institutions will participate in seminars and teaching programs, as well as co-supervise predoctoral students and postdoctoral fellows. To

date, the following internationally-known investigators have indicated a willingness to participate: Daniel Alkon, M.D., Chief of the Laboratory of Adaptive Systems at the NINDS (National Institute for Neurological Disease); Robert Desimone, Ph.D., Chief of the Section on Neurophysiology at the NIMH (National Institute for Mental Health); John Rinzel, Ph.D., Chief of the Mathematical Research Branch at the NIDDK (National Institute for Diabetes and Digestive and Kidney Diseases); Julius Axelrod, Ph.D., Nobel Laureate and Guest Worker at the Laboratory of Cell Biology at the NIMH (National Institute for Mental Health); and Shihab Shamma, Ph.D., Professor of Bioengineering at the University of Maryland.

SCIENTIFIC PROJECTS

1. Texture Analysis for Scene Segmentation

Over the past year Dr. Ledley and Mr. Frye during Phase I of their study (a) developed many of the software algorithms necessary for both calculating texture descriptor measurements and analyzing the ability of such descriptors to classify textures; (b) initiated the investigation of the utility of the Discrete Cosine Transformation (DCT) in texture recognition and classification; and (c) started to build a library of texture examples for testing and comparison of their methods.

Some of their recent results are outlined in a paper entitled "Texture Discrimination Using Discrete Cosine Transformation Based Shift-Insensitive Measurements." This manuscript is being prepared for submission to *IEEE Transactions on Pattern Analysis, Machine Intelligence* or *Pattern Recognition*. The article will describe how, among the numerous space-frequency based texture measurements, the Discrete Cosine Transformation (DCT) can be used to classify textures. The DCT, which extracts spatial-frequency (SF) components from a local image region, is the basis for the JPEG image compression standard and has many fast algorithmic implementations. By using a sliding DCT the group has derived a SF representation for a region of interest (ROI) surrounding

each image pixel. They have shown that the DCT coefficients may represent an SF as a combination of other SFs depending on the offset of the SF component maximum from the start of the ROI. Thus, the DCT coefficients for a texture with a given constant SF will change as the transformation is moved over the texture. In order to circumvent this problem, the group has derived horizontal and vertical shift-insensitive measurements of SF from DCT components which can be used in texture recognition. Examples are given which show how these DCT-based shift-insensitive measurements (DCTBSIMs) can be used to classify textured image regions. Since a large number of image display, storage and analysis systems are based on DCT hardware and software, DCTBSIMs may be easily integrated into existing technology.

Earlier work, which has been described in previous reports, and the group's current studies, have demonstrated that the DCT can be a useful tool in recognizing and classifying textures. They have applied this particular technique to the facial images from Environmental Research Institute of Michigan (ERIM), and it appears that their routines extract certain key features and that significant facial features can easily be extracted using their method. Dr. Ledley's group recognizes that only the application of segmentation or neural network algorithms will allow determination of the objective ability of these texture descriptors to extract facial features. Additionally, other methods of feature extraction should be compared to the performance of the DCT based texture measurements.

2. Mechanisms Underlying Cognitive Changes in Experimental Traumatic Brain Injury

Dr. Faden's research group has initiated several projects relating to effects of experimental brain trauma on cognitive function. Ongoing studies examine potential relationships between brain injury, learning and memory, and Alzheimer's disease (AD) on both a molecular and behavioral level. Epidemiological data indicate a connection between brain injury and early onset AD in

humans. A decrease in cp20, a novel G-protein expressed in fibroblasts and neurons, as well as a functional absence of the potassium current (I_K) which is regulated by cp20, are predictive of AD in humans. His laboratory is currently investigating whether brain injury induces changes in cp20 and potassium channels that resemble those associated with AD. Preliminary Western blot studies have been conducted which show that cp20 is detectable in the hippocampus and cortex of the rat in the early hours after fluid percussion-induced brain injury. Tissue samples prepared at 1, 4, 12, 24 h and 3 days after mild, moderate and severe injury have been prepared, which will be used to examine potential trauma-induced changes in cp20 mRNA and protein. An acute dissociation scheme for electrophysiological studies is being refined which will examine injury-associated changes in potassium channel conductance. These studies are being performed in collaboration with Dr. Rene Etcheberrigaray and Dr. Daniel Alkon at the NIH.

Behavioral work in progress examines learning and memory functions after brain injury using a Morris water maze paradigm. Several groups have demonstrated that experimental brain injury interrupts spatial memory consolidation when injury is induced within hours after training has been completed. However, planned studies are intended to address whether brain injury disrupts long-term spatial memory storage, as well as the ability to learn and recall new tasks. Preliminary data indicate that severe injuries are associated with lasting, profound deficits in acquisition of a new spatial task. Also under evaluation is the sensitivity of previously used motor assessments to learning and memory data obtained from the water maze, to assist in determining which tests are the best measures of injury-induced deficits. In the future, these tests will be used to assess pharmacological improvements in behavior by potential neuroprotective agents. Such agents may include antisense and/or ribozymes to cp20. Additionally, such learning/memory paradigms will be done in conjunction with a variety of NMR imaging/spectroscopy methods in collaboration with Dr. Pekar. Methods will include diffusion-weighted MRI ^{1}H and ^{31}P MRS, ^{17}O to evaluate changes in oxygen utilization and ^{19}F methods to examine changes in blood flow.

3. Cortical Mechanisms of Auditory Processing and Plasticity

Dr. Rauschecker's initial project seeks to explore the functional organization of multiple maps in the auditory cortex of higher mammals. His group is analyzing single unit responses to auditory stimuli in different auditory fields of cats and macaque monkeys. In addition to pure tones, standardized complex sounds are used for stimulation. Neurons in the anterior areas of the cat's auditory cortex (AAF and AEA) respond best to highly transient stimuli with a fast rate of frequency modulation (FM) and often display spatial tuning, which is experience-dependent. By contrast, neurons in the posterior areas (PAF, VPAF, DAF) tend to respond better to slow FM and are more sharply tuned to the rate of modulation. These results support a role for the anterior areas in the spatial analysis of sounds and for the posterior areas in auditory pattern recognition. In monkeys, a similar approach has been taken to explore the multiple maps in non-primary auditory cortex. Neurons in the rostro-lateral area (RL) respond preferentially to low-frequency sounds, as they are contained in communication signals, whereas neurons in the caudo-medial area (CM) prefer high frequencies, which are important for sound localization. A combination of lesioning and tracer injections into matched frequency representations reveals different inputs from the medial geniculate nucleus (MGN) of thalamus into areas RL and CM, with CM depending more on input from AI for its responsiveness. The lateral belt areas, which receive input from RL and AI, contain neurons that do not respond well to pure tones. Instead, these units can be driven briskly by bandpass-filtered noise pulses. Using this new type of stimulus, tonotopic maps can be revealed in three lateral areas. Neurons are tuned to the bandwidth of these stimuli, which is represented orthogonally to best center frequency. Noisy pulses are essential components of many species-specific communication sounds, and neurons in the lateral belt areas often respond specifically to such sounds as well. These areas may thus constitute a further stepping stone towards the analysis of auditory patterns, in particular those relevant for language evolution.

Although several maps have been identified in the auditory cortex, virtually nothing is known about their functional specialization, or how they are interconnected. Dr. Rauschecker's

group is therefore exploring the non-primary auditory cortex physiologically with standardized complex sounds generated by digital signal processing (DSP) techniques. Some of these stimuli contain essential components of natural vocalizations and some are spatially localized, the assumption being that the central auditory system is organized in similar dual fashion as a spatial and pattern system as found in the visual and somatosensory systems. His group will also combine this approach with the use of anatomical tracers in order to characterize the input-output connections of the cortical maps and relate them to their physiological response properties. These studies should ultimately lead to a better understanding of the central coding mechanisms in acoustic communication as well as localization used by the central nervous system. This should then allow a better understanding of certain forms of central deafness and, together with a better understanding of the mechanisms of cortical plasticity, may indicate novel treatment possibilities.

4. Development of Novel Drugs to Enhance Cognition and Probe Mechanisms of Cognitive Dysfunction

The research program in Dr. Kozikowski's laboratory includes the elucidation of novel chemical entities that may serve as therapeutics, or that can be used as pharmacological research tools for probing the connections between specific (brain) receptors and/or enzyme systems and physiological processes.

He had, for example, been engaged at the Mayo Clinic in efforts to discover more potent and selective chemical entities related to the cognition enhancing agent, huperzine A. Huperzine A is a naturally occurring alkaloid that acts as a potent and selective inhibitor of acetylcholinesterase. Huperzine A has also been shown to decrease neuronal cell death caused by glutamate in primary cultures derived from hippocampus and cerebellum of embryonic rat. Because of its dual pharmacological action, huperzine A would thus appear to be a particularly unique and important drug for the treatment of Alzheimer's dementia, as it can be used against both reduced ACh levels in the brain and neuronal cell death. With an aim toward advancing huperzine A, or one of its

analogues, to clinical use in this country, he has been engaged in a broader study to synthesize new analogues, to explore structure-activity relationships, and to examine the effects of structural changes on cognition enhancing properties. Recently, in collaboration with Dr. Sussmann of the Weizmann Institute, Dr. Kozikowski's group has succeeded in obtaining a huperzine A/acetylcholinesterase co-crystal, x-ray structure that permits the identification of the precise binding site of huperzine A within the enzyme. This information is believed to greatly facilitate their ability to design improved versions of this molecule.

During the coming six-month period, in addition to continuing the above described studies, efforts will be made to investigate pro-drug type modifications to the kappa-opioid antagonist nor-binaltorphimine (nor-BNI) in collaboration with Dr. Alan Faden's research group. These studies are being undertaken in order to improve upon the ability of nor-BNI to penetrate the blood-brain-barrier, thereby enhancing its possible use in the treatment of traumatic brain injury and associated cognitive impairment.

5. Optical Imaging, Distributed Processing, and Oscillatory Activity

Dr. Wu has research interests in three parallel areas: (a) the first aims to develop an optical recording apparatus in order to examine the initiation and propagation of excitatory events in mammalian brain slices; (b) the second seeks to understand the distributed processing in a small nervous system — Aplysia abdominal ganglion.; (c) the third examines the oscillatory activity in the pheromone sensing system of an oak moth.

a. The goal of this project is to image the electrical activity of the neurons with a rate rapid enough (c.a. 0.5 ms/frame) to follow the initiation and propagation of an epileptic event. To examine the spatial-tempo dynamics in a brain slice during epileptic events is an exciting topic because it suggests that a synchronized seizure may start from a chaotic event. Several laboratories are proposing similar experiments, but at the University of Maryland, Dr. Wu's group had optimized spatial and temporal resolution for the electrical events in epilepsy. During the last six

months they have completed the optical imaging apparatus and have done preliminary experiments to show complex patterns in the brain slice.

For the coming year, two kinds of experiments are proposed: the first will be to image the foci of several epileptic models. The second includes visualizing the functional change after local synapse hsa been altered by a genetically engineered viral vector (HSV). The HSV vector carries a glutamic acid decarboxylase (GAD) transgene which is expected to decrease glutamate levels and increase GABA levels in the infected area. This may reduce the excitability of the local circuitry and thus alter the initiation and spreading of an epileptic discharge. This work would contribute to the clinical epileptic research since GAD carrying HSV infection is considered to be a promising therapeutic approach for epilepsy.

b. Voltage-sensitive dyes and optical imaging will be used to monitor a large portion of neurons. This allows simultaneous monitoring of more than one-third of the neurons in a small system during different behaviors. Previous experiments have found that the same large population of neurons is activated during two different behaviors, suggesting that behaviors are generated by a distributed circuitry in the ganglion.

The goal is to understand how different behaviors are generated by the same large distributed circuit. During the last six months, Dr. Wu has examined the neurons activated during different behaviors such as the gill withdrawal reflex, respiratory pumping and other evoked neuronal events. He plans to examine the interaction between intrinsic and evoked behaviors in order to distinguish the common neuronal resources shared by different functions. He plans to modify the excitability of identified neurons during behaviors in order to study the roles of individual neurons in a large neuronal network. The distributed hypothesis, contrary to classical views, would shed new light to the understanding of how neurons interact to generate simple behaviors.

c. Oscillatory activities in the nervous system appear in almost all animals and systems. It is suggested that oscillations are fundamental in the nervous system although their physiological function remains largely unclear. Pheromone systems which process only a few odors are good

models for understanding the meaning of oscillations in a sensory system. During his last six months at the University of Maryland, Dr. Wu found three kinds of oscillations in the pheromone system of oak moth *Antheraea pernyi*, as well as three kinds of rhythmic activities in this system. The frequency of the oscillations differs with different stimuli. The next step of this work will be to image these oscillations and to examine how they can be modified by different sensory input.

6. Role of Protein Tyrosine Kinases in Neural Plasticity

Neurotransmitter receptors are of primary importance in synaptic transmission and are potential targets for regulation. Modulation of the function, expression, or density of receptors may have an effect on synaptic efficacy. Accumulating evidence supports a role for phosphorylation in the regulation of synaptic transmission. Protein tyrosine kinases are a unique class of kinases initially found to mediate cell transformation and proliferation. However, protein tyrosine kinases are also involved in differentiated cell function, and are in fact, highly expressed in the brain. They are present both pre- and postsynaptically, suggesting their importance in the regulation of synaptic activity. Dr. Swope's long-term goal is to study the role of protein tyrosine kinases in synapse formation and function.

Much of the current understanding of synapses originates from studies of the neuromuscular junction (NMJ). The nicotinic acetylcholine receptor (AChR) is the ligand gated ion channel that mediates rapid postsynaptic depolarization at the NMJ. Because of its abundance in the electric organs of *Torpedo californica,* the AChR is the best characterized neurotransmitter receptor and has served as a model to elucidate the structure, function, and modulation of neurotransmitter receptors and ion channels. The AChR is phosphorylated on tyrosine residues both *in vitro* and *in vivo*, and this tyrosine phosphorylation is correlated with a modulation of the rate of receptor desensitization. In addition, tyrosine phosphorylation of the AChR and/or other postsynaptic components is involved in the nerve induced clustering of the AChR during synaptogenesis at the NMJ. Their group's interest at Johns Hopkins University had been to

identify protein tyrosine kinases that are expressed postsynaptically at the NMJ which function to regulate the AChR.

At Johns Hopkins University, Dr. Swope's group identified two Src-like kinases, Fyn and Fyk, that together comprise the predominant protein tyrosine kinase activity in the AChR enriched postsynaptic membrane of Torpedo electric organ. Fyn and Fyk are also present in skeletal muscle and brain of *Torpedo* and mammals. In skeletal muscle, Fyn and Fyk activities appear to be regulated by differentiation and denervation. These two kinases associate with the AChR via a binding of their src homology 2 (SH2) domains to the tyrosine phosphorylated Î subunit of the receptor. In addition, Fyn and Fyk phosphorylate the receptor *in vitro*. These initial studies suggest that Fyn and Fyk are important regulators of synaptic function at the NMJ.

As a new laboratory at the Georgetown Institute of Cognitive and Computational Sciences, the goal of this group's current research is to continue to test the hypothesis that Fyn and Fyk are involved in synapse formation and function at the NMJ by regulating the AChR. Furthermore, the molecular mechanisms by which these two kinases act to regulate synaptic transmission will be investigated.

As a first step to attain their research goal, they plan to identify primary extracellular factors that regulate Fyn and Fyk activity. The ability of a series of known candidate factors including agrin, ARIA, and bFGF to stimulate Fyn and Fyk activity will be examined in muscle cells in culture and by using molecular biological and biochemical approaches. In addition, initial characterization of the signal transduction pathway(s) by which Fyn and Fyk act to regulate synapse formation and function will be begun by asking: what are the identities of cellular components that interact directly with Fyn and Fyk? These studies will be performed using co-immunoprecipitation techniques, fusion protein affinity chromatography, and the yeast two-hybrid system. A final short-term goal of the laboratory is to molecularly clone the mammalian homologue of Torpedo Fyk, a novel protein tyrosine kinase. The mammalian Fyk and Fyn clones will used for subsequent investigation of the physiological roles of these two protein tyrosine kinases in the regulation of AChR phosphorylation, clustering, expression, and channel kinetics.

Clarification of these basic molecular processes are relevant to an understanding of synaptic transmission in healthy people as well as those afflicted with neurological and neuromuscular diseases.

7. Experimental Studies of Alzheimer's Disease

Dr. Etcheberrigaray's interest in Alzheimer's disease (AD), derives from his previous appointment at the NIH where he was involved in the study of molecular mechanisms of associative memory in both invertebrates and vertebrates. Initial observations in the marine snail *Hermissendu*, later confirmed in rabbits, revealed a cascade of events resulting in the long term reduction of specific potassium (K^+) currents in identified neurons with the acquisition of a conditioned response. The events involved in this reduction include key elements such as Protein Kinase C activation, phosphorylation of the GTP-binding protein Cp20, and 1P3 mediated calcium (Ca^{+2}) mobilization.

Alzheimer's disease characteristically exhibits memory loss early in the course of the disease. Thus, his research at the NIH explored the possibility that the molecular mechanisms of associative memory may be altered in AD. Several laboratories had previously found alterations in peripheral cells from AD patients, indicating that AD may have systemic expression. In addition, peripheral cells, such as fibroblasts, allow the exploration of molecular changes in AD patients without interference of the pathological process which occurs in the brain. Fibroblasts from AD patients did indeed exhibit alterations in K^+ channels, Ca^{+2} mobilization, and Cp20 was found to be absent or significantly reduced in fibroblasts from AD patients. Furthermore, some of these particular alterations could be induced in normal fibroblasts by incubating the cells with 10 nM β-amyloid, a protein thought to be involved in the pathogenesis of AD.

PROJECTS to be developed at GICCS include the following:

a. Pathophysiological Mechanisms in AD. Peripheral and brain tissue will be used to study the effects of β-amyloid and related peptides on ion channel function and intracellular calcium

handling. The main goal is to identify the precise mechanisms by which β-amyloid alters cellular function, and how relevant those actions are in the context of the disease process and its clinical manifestations.

b. The Study and Description of Cellular and Molecular Alterations in AD. In addition to the previous memory-related molecular alterations in AD, numerous other alterations have been described in cells from AD patients. Dr. Etcheberrigaray's research laboratory will study the mechanistic relationship between these multiple alterations in order to identify those directly linked to the pathophysiology of the disease.

8. Development of a Nuclear Magnetic Resonance (NMR) Research Laboratory and Program

The GICCS NMR Laboratory, housed in 2,400 square feet of space in the basement of the new Research Building, will feature a 7.0 Tesla magnetic resonance rodent scanner — the highest-field MR scanner in the greater Washington area. The scanner will be capable of multidimensional multinuclear imaging and spectroscopy, reporting on many aspects of brain physiology and function. This will include "*in vivo* histology and biochemistry," or the ability to characterize tissue pathophysiology in a non-invasive manner.

Design of the laboratory is complete, and construction has begun. Completion of construction and delivery of the scanner are scheduled for December 1995, and the scanner should be fully operational in early 1996. Other facilities of the NMR laboratory will include: an animal physiology lab; an electronics shop; computer facilities; and office space for staff.

Recruitment of laboratory personnel is in progress. Dr. Pekar continues collaborative research at the National Institutes of Health, where he maintains an affiliation with the Laboratory of Diagnostic Radiology Research. His work there focuses on non-invasive methods for mapping brain physiology and function. Recent progress includes development of methods for whole-brain

investigative human brain mapping using blood oxygen level dependent functional MRI (BOLD fMRI), and quantitative methods for mapping cerebral blood flow in both animals and humans.

9. Reading Disorders in Neurological Disease

Continuing her association with the Georgetown University Medical Center's Department of Neurology, Dr. Friedman has two major research projects: one investigates reading, writing and phonologic processing in patients with Alzheimer's Disease (AD), and the other evaluates experimental therapies for acquired reading disorders (acquired dyslexias) in stroke patients, based upon a cognitive neuropsychological model of reading.

Findings in the first study had indicated that patients with AD have remarkably well preserved processing of orthographic information. This contrasts with a subtle but significant decline in phonologic processing, even for mildly demented patients. Additionally, problems with reading certain "mildly irregular" words may be attributable to the AD patients' inability to suppress activation of competing pronunciations generated by subword units with multiple correspondences.

Results from studies involving experimental treatments for acquired dyslexias failed to find evidence for an ability of pure dyslexic patients to read via semantics, contrary to predictions made by others in the literature. Nevertheless, it was found that patients with pure dyslexia can be retrained to recognize words very rapidly, indicating that something other than purely visual characteristics has been learned. However, this finding does not generalize to other words. It was also demonstrated that patients with deep dyslexia may be able to learn to decode written words using syllables, which are psychologically real, as building blocks, rather than phonemes. As a result of this project, a new form of dyslexia, phonologic text dyslexia, has been identified. This form of dyslexia, which is hypothesized to be the result of rapidly decaying phonologic codes, affects the reading of sentences but not of single words.

Each of the above projects will continue in the next year. In addition, a new study will begin that involves the use of functional MRI (fMRI) in the stroke patients who are participating in the dyslexia treatment study. Functional MRI scans of the brains of normal controls will be taken while the subject silently reads words of various types. Patients who have been diagnosed with a specific type of dyslexia will receive fMRI scans of the brain prior to beginning a treatment protocol, and again upon completion of the treatment. Data will be examined for differences in the brain scans of dyslexic patients compared with normal subjects who are performing the same word reading task to help localize the source of the reading deficit. Scans taken on the same patient pre- and post- treatment will be compared to help elucidate the mechanism of recovery, when improvement is noted. Pre-treatment scans of patients who respond well to a treatment will be compared with scans of patients who fail to respond to the same treatment; differences may point to data in the pre-treatment scans that can serve as predictors of success of the respective treatment. When possible, post-treatment scans of patients who show similar pre-treatment scans will be compared, particularly when the patients receive different treatments.

10. Audition and Speech Processing

In the past year at the NIH, Dr. Tian's research has focused on auditory processing in the rhesus monkey cortex, which provides an excellent model system for the study of human audition and speech processing. Although several physiological areas have been identified in the monkey auditory cortex, virtually nothing is known about their functional specialization or their interconnections. His group has, therefore, begun to explore the auditory cortex using a combination of physiological and anatomical methods.

At the NIH Dr. Tian recorded single-unit activity in the newly discovered lateral belt areas of the rhesus monkey auditory cortex. To explore the functional specialization of these areas he used frequency-modulated (FM) sounds as stimuli in anesthetized animals. He found that neurons in different areas preferred different FM rates. Since many monkey vocalizations contain FM

sounds, and the preferred FM rates of neurons in lateral belt areas are well in the range of FM rates in monkey vocalizations, these areas may be involved in the processing of monkey vocalizations.

Dr. Tian also recorded from awake rhesus monkeys using a variety of acoustic stimuli including FM sounds and monkey vocalizations. Preliminary data confirmed these findings in anesthetized animals. Spectral combination-sensitive and temporal combination-sensitive neurons were also found in the awake animal. These neurons are of special interest because they may be the neural substrate for decoding communication sounds. At present, he is training a monkey to perform auditory behavioral tasks in order to study the neural basis for auditory attention and memory. Once the monkey is trained, single-unit activity will be recorded in the lateral belt areas using different acoustic stimuli.

In collaboration with colleagues at the NIH, Dr. Tian also performed functional anatomy experiments. His group mapped the belt areas and injected anterograde and retrograde tracers to study neuroanatomical projection patterns. The brains are currently being processed and analyzed.

At GICCS Dr. Tian will continue electrophysiological and anatomical studies on the monkey auditory cortex in both anesthetized and awake animals. Since only one or a few neurons at a time can be recorded with single-unit electrophysiology, the laboratory will establish an optical-imaging system to give them unparalleled insight into the ensemble of neuronal activity in the auditory cortex.

11. Molecular Studies of Neuroplasticity in Brain Injury

Dr. Yakovlev's major research focus has been the molecular basis of kappa-opioid receptor (KOR) heterogeneity and the role of KOR-based signaling in the regulation of cognitive function, as well as on neuron plasticity after brain injury. He intends to continue work in these areas. At. Georgetown University Medical Center, Dr. Yakovlev was the first to clone and characterize the gene encoding KOR with its entire promoter region. Cloning experiments provided information about the genomic organization of the KOR system, and did not show evidence of other genes highly homologous to KOR. Despite the apparent lack of gene

heterogeneity or alternative splicing, he discovered a mechanism that could give rise to potentially different KOR species. The finding of two KOR mRNA species indicates that alternative promoters may be operational in the transcriptional regulation of KOR gene.

Dr. Yakovlev is also involved in studies on endogenous autodestructive and neuroprotective factors in traumatic CNS injuries, which are designed to clarify molecular mechanisms of secondary tissue damage and neuroplasticity. In these studies he uses RT-PCR, Northern blot analysis, subtractive hybridization and differential display techniques to estimate dynamic changes in transcriptional activity of genes encoding for a number of factors — protooncogenes, cytokines, opioid peptides, growth factors, adhesion molecules and genes involved in a programmed cell death (apoptosis). Experiments are performed in rat and subsequently mouse models of brain trauma as well as for *in vitro* injury.

CONCLUSIONS

Although the start-up phase of the GICCS was delayed by the need to complete research laboratories in the NRB and a lengthy search for an Institute Director, following Dr. Faden's appointment to lead the project, there has been rapid development of the Program. Nine new faculty have been recruited, with an additional five or six faculty to be recruited by next summer. Substantial recruitment of research support staff has also been accomplished. Laboratory equipment has been purchased and laboratories modified to meet the requirements of faculty researchers. The Office of the Director is now fully staffed with a full-time Administrator and Medical Secretary. During this transition period, collaborative research by faculty has continued. We are confident that the next 12 months will show substantial research productivity in the Institute's areas of research emphasis.